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DIALYSIS – TRANSPLANTATION

Bicarbonate/lactate-based peritoneal dialysis solution increases cancer antigen 125 and decreases hyaluronic acid levels

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Bicarbonate/lactate peritoneal dialysate increases cancer antigen 125 and decreases hyaluronic acid levels.

Background. In a randomized, controlled trial comparing a pH neutral, bicarbonate/lactate (B/L)-buffered PD solution to conventional acidic, lactate-buffered solution (C), the overnight dialysate levels of markers of inflammation/wound healing [hyaluronic acid (HA)], mesothelial cell mass/membrane integrity [cancer antigen 125 (CA125)], and fibrosis [transforming growth factor-β1 (TGF-β1) and procollagen I peptides (PICP)] were assessed over a six-month treatment period.

Methods. One hundred six patients were randomized (2:1) to either the B/L group or C group. Overnight effluents were collected at entry into the study (time = 0 all patients on control solution) and then at three and six months after randomization. Aliquots were filtered, stored frozen, and assayed for HA, CA125, TGF-β1, and PICP. Differences between groups were assessed by repeated-measures analysis of variance for unbalanced data using the SAS procedure MIXED.

Results. In patients treated with B/L, there was a significant ($P = 0.03$) increase in CA125 after six months compared with time = 0 (19.76 ± 11.8 vs. 24.4 ± 13.8 U/mL; mean ± SD; $N = 51$). In the same group of patients, HA levels were significantly decreased at both three and six months in the B/L-treated group (time = 0, 336.0 ± 195.2; time = 3 months, 250.6 ± 167.6; and time = 6 months, 290.5 ± 224.6 ng/mL; mean ± SD; $P = 0.006$, $N = 47$ and $P = 0.003$, $N = 48$, respectively). No significant changes in CA125 or HA levels were observed in the control group. There were no significant changes observed in the levels of PICP or TGF-β1 in the B/L or C group over the six-month treatment period.

Conclusions. These results suggest that continuous therapy with the B/L solutions modulates the levels of putative markers of peritoneal membrane integrity and inflammation. In the long term, this may positively impact the peritoneal membrane, increasing its life as a dialyzing organ.

Peritoneal dialysis (PD) has been successfully employed as a form of renal replacement therapy for over 20 years. During the last decade, numerous in vitro and in vivo reports have demonstrated that conventional lactate-buffered PD solution formulations have bioincompatible characteristics [1, 2]. These are related to their acidity, buffer composition, glucose content, and hyperosmolality [3–6]. Despite this evidence of its bioincompatibility, acidic lactate-buffered PD fluids (PDFs) are still used in the vast majority of PD patients.

One of the major inhibitory pathways by which conventional PD solutions modulate cell function is related to the combination of their acidity and lactate concentration [4, 7]. To address this issue, the introduction of bicarbonate, the body's natural buffering system, which would allow the creation of a neutral pH solution, was considered desirable [8]. Its introduction was, however, initially hampered by the problem of calcium carbonate precipitation at the bicarbonate levels required to correct metabolic acidosis in renal failure [9]. The development of new container systems has permitted the use of bicarbonate as a buffer system for PD. A two-chambered design allows the bicarbonate and calcium to be sepa-

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rated during the sterilization process, thus avoiding precipitation of calcium carbonate [10, 11]. In addition, the glucose compartment is maintained at a pH value during sterilization and storage, which diminishes the degree of glucose degradation product formation [12]. Because the two chambers are mixed immediately prior to use, this system allows the infusion of a bicarbonate-based solution at neutral pH with reduced levels of glucose degradation products.

In vitro studies with bicarbonate-based formulations have consistently shown improvements in biocompatibility parameters over acidic, lactate-based solutions. In addition, animal infusion models have shown that peritoneal mesothelial morphology is also better preserved following repeated solution infusion [reviewed in 13]. While this large body of experimental data provides indirect evidence for the potentially improved biocompatibility of bicarbonate-buffered solutions, few data are available on their impact on the peritoneum in vivo. Previous clinical trials with various bicarbonate formulations have demonstrated that they are well tolerated and clinically effective [10, 11, 14–17].

To assess the in vivo impact of different infused solutions on parameters related to peritoneal host defense, we and others have used an ex vivo approach to measure peritoneal macrophage (PMØ) function [6, 18]. Using this type of study, we have recently reported that PMØ function in patients exposed to pH neutral bicarbonate- and particularly bicarbonate/lactate (B/L)-buffered solutions was superior [as assessed by ex vivo tumor necrosis factor-α (TNF-α) synthesis] than that seen in cells exposed in vivo to acidic lactate-buffered solutions [19, 20].

While ex vivo cell function studies provide evidence for modulation of cellular function in vivo, there is a growing interest in the measurement of potentially prognostic markers of the structural and functional changes that occur in the peritoneal environment during PD [21–26]. In this respect, the effluent concentrations of markers of mesothelial cell integrity/mass, inflammation, fibrosis, and angiogenesis have been evaluated in various groups of PD patients [21–26].

In the present study, effluent concentrations of cancer antigen 125 (CA125), hyaluronic acid (HA), transforming growth factor-β1 (TGF-β1), and procollagen I C-terminal peptide (PICP) were evaluated. CA125 is used as a marker of ovarian neoplasms but has subsequently been suggested as a marker of mesothelial cell mass/turnover [27]. HA has been suggested as a marker of inflammation and tissue remodeling in the peritoneal cavity [21, 28]. In PD patients, HA levels appear to increase with time on PD suggestive of ongoing inflammation, wound healing, or mesothelial cell repair processes [21]. TGF-β1 and PICP were selected as potential markers of fibrosis. TGF-β1 is the prototypical cytokine associated with fibrogenesis in many disease processes throughout the body, capable of inducing extracellular matrix expansion and collagen deposition [29]. PICP and procollagen III N-terminal peptide (PIIICP) have been used as indirect measures of collagen turnover in various tissues, including the peritoneal cavity [26, 30–32].

Our results demonstrate that patients continuously exposed to B/L-buffered solutions exhibited significantly elevated effluent levels of CA125 and reduced levels of hyaluronan in their overnight effluent. These observations suggest reduced proinflammatory potential and improved mesothelial cell integrity within the dialyzed peritoneum, possibly indicating better preservation of peritoneal homeostasis and the long-term function of the peritoneal membrane in patients dialyzed with more biocompatible solutions.

METHODS

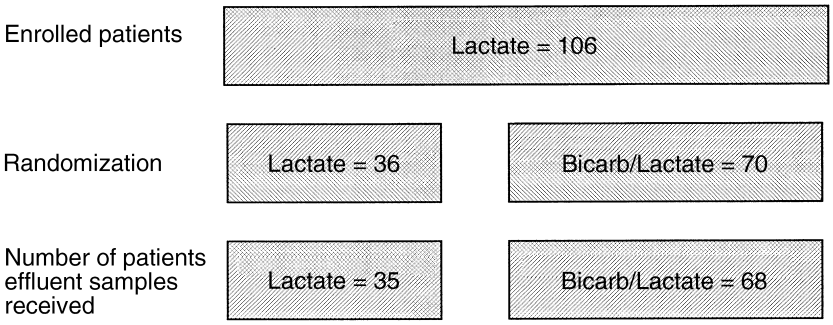
Study design and test solutions

This study formed part of a randomized, prospective, controlled, multicenter study comparing a new 25 mmol/L bicarbonate/15 mmol/L lactate (B/L) with a standard 40 mmol/L lactate-buffered PD (C) solution over a six-month treatment period [33]. For an initial run-in phase of four weeks, all patients received their standard dialysis regimen using the conventional PD solution (Dianeal PD4; 40 mmol/L lactate, pH 5.3 to 5.5 containing 1.36, 2.27, or 3.86% dextrose as appropriate; Baxter Healthcare, Deerfield, IL, USA). After this run-in period, patients were randomized in a 2:1 ratio to either B/L or control solutions for the trial period. All patients received their standard dialysis regimen using either C or B/L containing either 1.36, 2.27, or 3.86% dextrose. The electrolyte concentrations of each solution were identical [19]. The study design is shown in Figure 1.

Patients

This study took place in 17 centers in Europe [33]. A total of 106 patients were initially recruited, of which 70 were randomly allocated to B/L and 36 to the control group. Each patient gave written informed consent prior to participation, and the local ethics committee for each center gave approval for the study. Effluent samples were received from 68 patients in the B/L group and from 35 patients in the control group. The baseline demographics in the two patients groups were not statistically different nor was the time on dialysis prior to the commencement of the study (Table 1). The clinical changes occurring in the two patient groups are reported elsewhere [33].

Prior to entry in the study, patients had been on continuous ambulatory PD (CAPD) for at least three months and were treated with a 40 mmol/L lactate dialysis solution (Dianeal® PD4) using an integrated disconnect system (Twin-bag) for at least one month. They had to have a normalized (to body surface area) glomerular filtration



Effluent samples received and analyzed

	CA125	HA	TGF	Coll	CA125	HA	TGF	Coll
0 time	**34**	**30**	**31**	**30**	**65**	**52**	**54**	**55**
Not collected/dropout	1/0	5/0	4/0	5/0	2/1	15/1	13/1	12/1
3 months	**31**	**30**	**30**	**30**	**53**	**47**	**50**	**47**
Not collected/dropout	1/3	2/3	2/3	2/3	3/12	9/12	6/12	9/12
6 months	**26**	**25**	**26**	**24**	**51**	**48**	**52**	**50**
Not collected/dropout	2/7	3/7	2/7	4/7	4/13	7/13	3/13	5/13

Fig. 1. Flow diagram of patients and sample collection and measurement during the study.

Table 1. Baseline demographics

Demographic variables	Patient group: Lactate $N = 35$	Patient group: Bicarbonate/lactate $N = 68$	P value
Age *years*	57.4	55.8	0.4711
Range (min-max)	23.6–76.6	26.0–77.3	
Body weight *kg*	75.2	71.6	0.2230
Range (min-max)	52.8–124.0	44.8–103.0	
Height *cm*	169.0	168.8	0.8263
Range (min-max)	153–187	151–186	
Males	18 (51.4%)	41 (60.3%)	0.3890
Primary renal disease			0.4093
Glomerulonephritis	8	15	
Diabetic nephropathy	3	4	
Hypertensive nephropathy	3	12	
Polycystic kidney disease	7	5	
Interstitial/obstructive	3	8	
Other	11	24	
Diabetic type	7	7	0.2861
Type 1	2	5	
Type 2	5	2	
Normalized RRF	2.92	2.83	0.8508
Weekly total Kt/V	2.20	2.26	0.4729
Weekly normalized creatinine clearance *L/week/1.73 m²*	75.03	76.50	0.6404
Mean time on dialysis at the start of the study	20.15	22.56	0.6863

rate of ≤7 mL/min/1.73 m² (nRRF, average of renal and creatinine clearance) and used four or five, 2 to 2.5 L exchanges a day for seven days a week with no dry period. Their total weekly creatinine clearance (peritoneal and renal) had to be ≥55 L/1.73 m² body surface area, as determined by the PD Adequest® program (version 1.4a; Baxter Healthcare). Patients were excluded if they had an acute or chronic exit site or tunnel infection, had completed a course of antibiotics for exit site/tunnel infection or for peritonitis in the previous 30 days, or had other serious illnesses, including the need for hospitalization in the previous 30 days. Patients were also excluded if they were known to be HIV positive, were pregnant or lactating, were adding bicarbonate to their bags, or were taking bicarbonate orally.

By six months, 55 patients remained in the treatment group, and 29 remained in the control population. Withdrawals occurred because of transplantation (7 patients, 4 controls and 3 B/L), adverse events such as peritonitis (9 patients: 3 controls, and 6 B/L) and unavailability of

treatment options (bag size, night exchange) with the experimental B/L product (4 patients).

Effluent collection

At the end of the four-week run-in phase and prior to randomization (time = 0) and at time = 3 months and time = 6 months, peritoneal effluent was collected from a timed overnight (18 hours) dwell using a 1.36% dextrose solution appropriate to the patient's randomization. Approximately 50 mL of overnight (18 hours) drain fluid were collected, rendered cell free by filtration (0.4 μ filter; Millipore UK, Ltd.) and then aliquoted into 5 mL cryovials (Nunc, Life Technologies Ltd., Paisley, UK) for storage at −70°C prior to assay in a single laboratory. Actual effluent samples received for analysis from each sampling time period are shown in Figure 1. Reasons for missing samples were classified into patient dropout or not collected.

Peritoneal effluent markers

Transforming growth factor-β1. TGF-β1 was measured following acid activation of samples by enzyme-linked immunosorbent assay (ELISA; Amersham-Pharmacia Biotech UK Ltd., Amersham, UK) as per the manufacturer's instructions. The minimal detectable concentration in the assay was 4 pg/mL, and the coefficient of variation for intra-assay and interassay variability was between 3.0 and 4.0% and 7.7 to 13.4%, respectively.

Procollagen I peptides. PICPs were measured by ELISA (Prolagen C assay; Metrabiosystems Inc., Sunnyvale, CA, USA) as per the manufacturer's instructions. The minimum detectable concentration in the assay was 0.2 ng/mL, and the coefficient of variation for intra-assay and interassay variability was between 5.5 and 6.8% and 5.0 to 7.0%, respectively.

Hyaluronic acid. HA was measured by commercial ELISA (Hyaluronic Acid "Chugai" Quantitative test kit; TCS Biological Ltd., Bucks, UK) according to the manufacturer's instructions. The minimum detectable concentration in the assay was 10 ng/mL and the coefficient of variation for intra-assay and interassay variability was between 3.6 and 4.2% and 5.7 to 7.0%, respectively [34].

Cancer antigen 125. CA125 was measured with the IMx CA125 microparticle enzyme immunoassay (Abbot IMx; Abbot Laboratories, Chicago, IL, USA) as per the manufacturer's instructions.

Statistical analysis

All effluent samples were archived centrally and then shipped for analysis, in a blinded fashion, to Cardiff or Amsterdam. The data generated were collated, and statistical evaluations were performed as described in this article. The variability estimates for all parameters measured increased when the respective means increased. This indicated that the data were highly skewed and therefore log transformed for analysis. We used log transformation to normalize the data and stabilize the variances. As the data were serial over time within patients but unbalanced data across patients, we analyzed the data by repeated-measures analysis of variance (ANOVA) for unbalanced data using the SAS procedure MIXED [35–37]. This was performed separately for each group (C and B/L). A value of $P < 0.05$ was considered statistically significant.

RESULTS

Significant variability was seen over time for all patients irrespective of the peritoneal markers in question (Figs. 2–5). There was no correlation between these differences and patient group characteristics, such as age or previous time on dialysis, emphasizing the requirement to compare the trial phase to run-in, thereby using the individual patients as their own controls.

Cancer antigen 125

Repeated-measures ANOVA comparing data from baseline (time = 0) and trial phases within each treatment group revealed a significant increase in effluent CA125 levels after six months continuous treatment with B/L ($P = 0.0274$; Fig. 2). Median (min-max) CA125 levels (U/mL) increased from 16.73 (3.36 to 61.5) at time = 0 to 17.64 (2.88 to 61.07) at three months ($P = 0.0931$, $N = 53$) and 21.23 (5.9 to 63.8) after 6 months ($P = 0.0274$, $N = 51$). In the control group, median CA125 levels did not change between the run-in and the trial phases of the study. At time = 0, three and six months CA125 levels (U/mL) were 14.44 (4.3 to 85.2), 14.0 (3.5 to 64.29, $P = 0.707$, $N = 31$), and 15.9 (1.32 to 50.85, $P = 0.792$, $N = 26$), respectively.

Hyaluronic acid

A comparison of HA levels (ng/mL) in timed overnight effluent between baseline (time = 0) and trial phases identified a significant reduction in its levels (at both 3 and 6 months) in the B/L group (Fig. 3). Median (min-max) levels were 305.0 (63.35 to 1095.0) at time = 0, which decreased significantly after three months to 213.15 (11.04 to 757.0, $P = 0.0058$, $N = 47$) and at six months to 243.2 (55.05 to 1382.2, $P = 0.0033$, $N = 48$) in those individuals dialyzed with B/L. In the control group, HA levels did not change significantly between the run-in and trial phases of the study. At time = 0, three- and six-month HA levels (ng/mL) were 278.4 (30.05 to 780.5), 238.6 (40.9 to 844.6) ($P = 0.69$, $N = 30$), and 316 (88.25 to 912.14, $P = 0.77$, $N = 25$), respectively, for patients continuously treated with acidic lactate-buffered solutions.

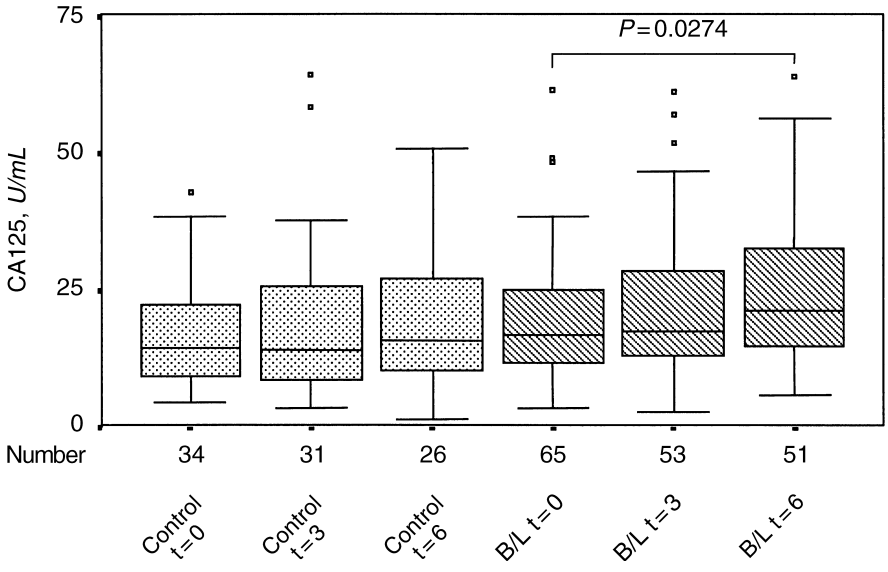


Fig. 2. Effect of continuous treatment with control (PD4, 40 mmol/L lactate, pH 5.2) or B/L (25 mmol/L bicarbonate/15 mmol/L lactate, pH 7.3) on the changes in effluent cancer antigen 125 (CA125) levels in overnight timed dwell effluents. Data are presented as median (dark line), range (box), and 95% CI (error bars) after the run-in phase (t = 0 all patients treated with control solutions) and the trial phase [at 3 and 6 months (t = 3 and t = 6) in patients randomized to control or B/L solutions]. *N* represents the number of patients in each group. Data comparisons were made with repeated-measures ANOVA.

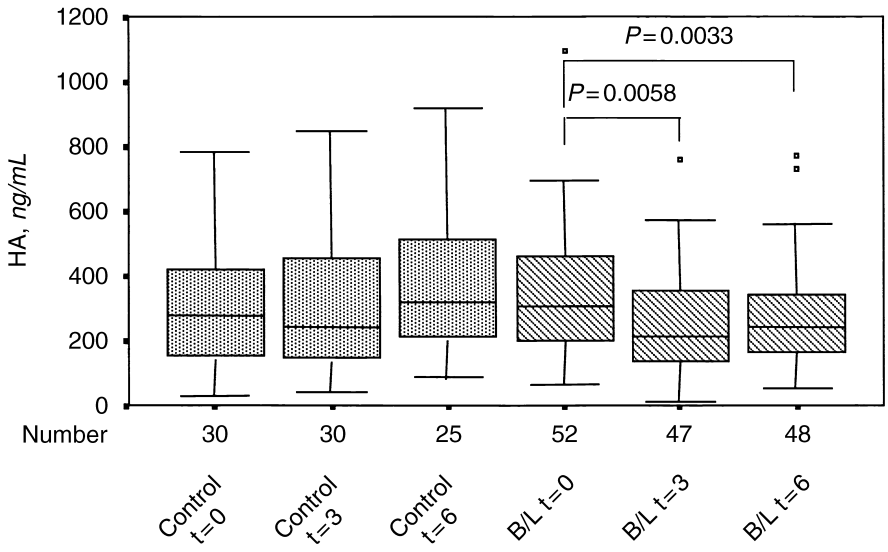


Fig. 3. Effect of continuous treatment with control (PD4, 40 mmol/L lactate, pH 5.2) or B/L (25 mmol/L bicarbonate/15 mmol/L lactate, pH 7.3) on the changes in effluent hyaluronic acid (HA) levels in overnight timed dwell effluents. Data are presented as median (dark line), range (box), and 95% CI (error bars) after the run-in phase (t = 0 all patients treated with control solutions) and the trial phase [at 3 and 6 months (t = 3 and t = 6) in patients randomized to control or B/L solutions]. *N* represents the number of patients in each group. Data comparisons were made with repeated measures ANOVA.

Transforming growth factor-β1

Analysis of the TGF-β1 levels in the C and B/L groups revealed no statistically significant changes in either group (Fig. 4A). Median (min-max) levels in the B/L group were ng/mL 83.21 (23.6 to 464.4), 77.52 (25.87 to 395.7, $P = 0.27$, $N = 50$), and 88.67 (22.87 to 429.1, $P = 0.48$, $N = 52$) at time = 0, time = 3 months, and time = 6 months, respectively. In the control group, median (min-max) values were 85.03 (23.6 to 433.0), 85.05 (28.12 to 374.09, $P = 0.69$, $N = 30$), and 93.06 (27.37 to 474.2, $P = 0.51$, $N = 26$) at time = 0, time = 3 months, and time = 6 months, respectively.

Procollagen I peptides

Analysis of the PICP levels in the control and B/L groups revealed no statistically significant changes in either group (Fig. 5A). Median (min-max) levels in the B/L group were ng/mL 286.6 (93.7 to 486.4), 318.2 (106.7 to 504.3, $P = 0.48$, $N = 47$), and 245.2 (107.5 to 422.85, $P = 0.0.14$, $N = 50$) at time = 0, time = 3 months, and time = 6 months, respectively. In the control group, median (min-max) values were 254.9 (107.1 to 699.9), 246.88 (59.3 to 860.8, $P = 0.58$, $N = 30$), and 252.29 (98.9 to 819.3, $P = 0.76$, $N = 24$) at time = 0, time = 3 months, and time = 6 months, respectively.

DISCUSSION

To date, the vast majority of biocompatibility studies have involved in vitro assessment of cell functional parameters following various periods of exposure to different PD solutions [reviewed in 2, 38]. These studies have

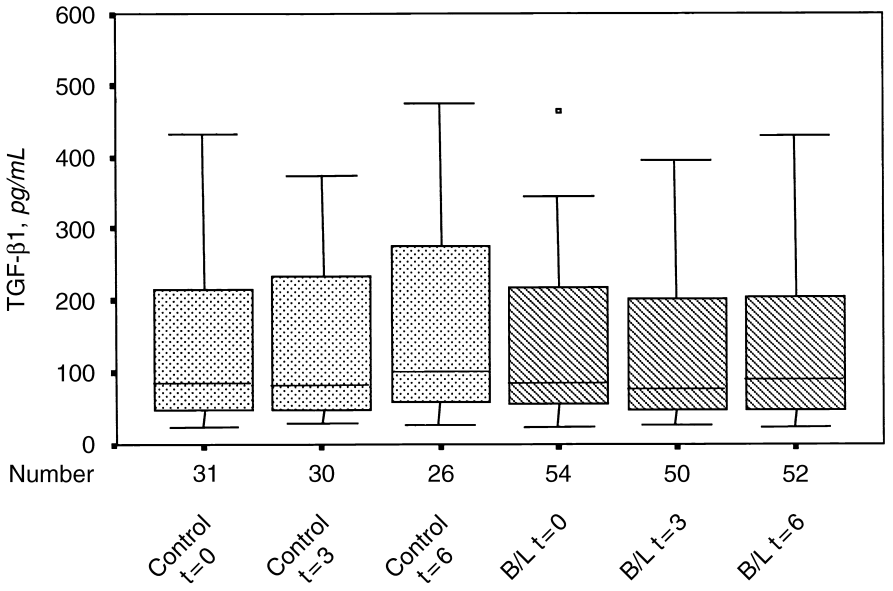


Fig. 4. Effect of continuous treatment with control (PD4, 40 mmol/L lactate, pH 5.2) or B/L (25 mmol/L bicarbonate/15 mmol/L lactate, pH 7.3) on the changes in effluent TGF-β1 levels in overnight timed dwell effluents. Data are presented as median (dark line), range (box), and 95% CI (error bars) after the run-in phase (t = 0 all patients treated with Control solutions) and the trial phase [at 3 and 6 months (t = 3 and t = 6) in patients randomized to control or B/L solutions]. *N* represents the number of patients in each group. Data comparisons were made with repeated-measures ANOVA.

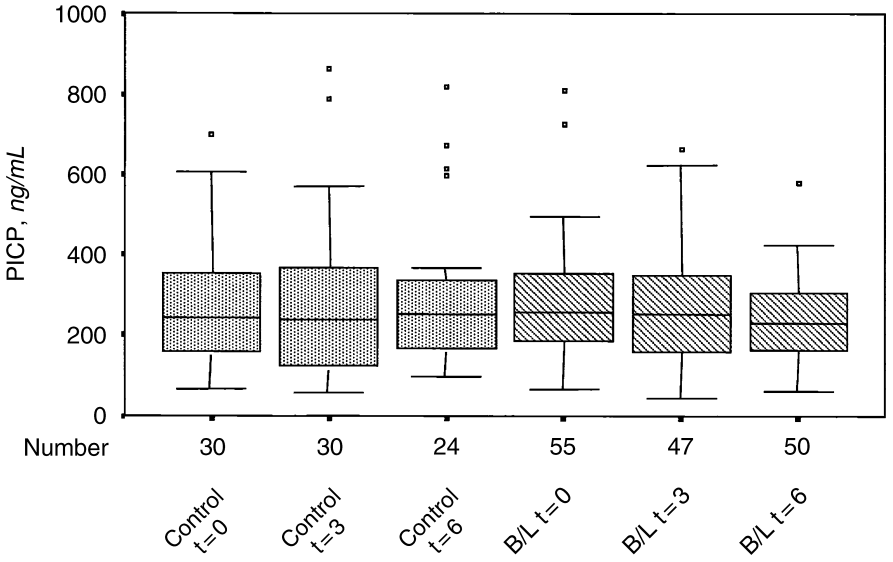


Fig. 5. Effect of continuous treatment with control (PD4, 40 mmol/L lactate, pH 5.2) or B/L (25 mmol/L bicarbonate/15 mmol/L lactate, pH 7.3) on the changes in effluent procollagen I peptide (PICP) levels in overnight timed dwell effluents. Data are presented as median (dark line), range (box), and 95% CI (error bars) after the run-in phase (t = 0 all patients treated with control solutions) and the trial phase [at 3 and 6 months (t = 3 and t = 6) in patients randomized to control or B/L solutions]. *N* represents the number of patients in each group. Data comparisons were made with repeated-measures ANOVA.

identified the potential of solution components to modulate cell behavior; however, they are not able to offer information on the in vivo response of the peritoneal cavity to conventional or newer dialysis solution components during long-term clinical dialysis. The response of the peritoneum to dialysis in vivo has been observed directly from peritoneal biopsy samples obtained cross sectionally from PD patients (abstract; Topley et al, *J Am Soc Nephrol* 10:324A, 1999) [39–48]. While these studies have identified that significant interstitial and vascular changes occur in the peritoneal membrane (with time on PD), they have not as yet identified whether specific dialysis solution components contribute to the described histologic alterations, although it is widely assumed that they play a significant role in this process (abstract; *ibid*) [40, 41, 49].

Insight into the effects of specific solution components is available from animal studies in which dialysis solutions or their components have been infused repeatedly, and their effects on peritoneal and mesothelial cell morphology have been evaluated [50–53]. In humans, the majority of data available on the in vivo effect of solution components is derived from ex vivo measurements of cell function. These experiments usually assess cell function in PMØ isolated from drain effluent after varying in vivo dwell times [6, 18–20, 54]. To date, the findings of these studies have largely mirrored in vitro observations and are suggestive of improvements in function in

cells exposed to potentially more biocompatible solutions such as those buffered with bicarbonate or B/L mixtures [13, 19, 20, 55–58]. While these studies appear to indicate the potentially beneficial effects of more biocompatible solutions on both peritoneal cell function and host defense against infection, they do not in themselves provide any direct information about the status (or changes) of the peritoneal membrane during ongoing dialysis, and neither can they be used as prognostic markers of adverse changes occurring over time or in response to infection during PD.

Over the past decade, interest has increased in the identification of potential markers that can be measured in the available peritoneal drain effluent that might provide an indication of the overall status of the peritoneum in vivo [59, 60]. These markers also might represent candidate biocompatibility markers, changes in which being indicators of the host's long-term response to different dialysis solutions (components thereof) or uremia per se [49]. To date, several candidate markers have been identified based on their relationship to changes in inflammatory status, mesothelial cell integrity, and the processes of wound healing and fibrosis [21–23, 25–27].

In the present study, we evaluated the impact of continuous dialysis with a potentially more biocompatible, neutral pH solution formulation buffered with a mixture of bicarbonate and lactate on changes in these parameters in a large group of CAPD patients [33]. Continuous dialysis with B/L was associated with significantly elevated CA125 levels after six months of treatment. This 220 kD glycoprotein, a marker of ovarian neoplasms but of unknown function, was first suggested by Visser et al as a putative marker of mesothelial cell mass/turnover [27]. These authors were able to demonstrate its constant secretion by cultured human mesothelial cells, which was apparently unaffected by known proinflammatory activators. While this has been disputed by others [61, 62], there is a general consensus that despite the limitations of previous cross-sectional studies [23, 25], its constant secretion rate in vivo and its cell specificity (only secreted by mesothelium) indicate that longitudinal changes in this parameter (in individuals with baseline historic values) may be a marker of the viability/status of the mesothelium [49]. The mesothelium plays a pivotal role in the peritoneum's response to inflammation and tissue injury [63, 64]. During PD, continuous mesothelial injury occurs, as evidenced by increased numbers of exfoliated cells present in dialysis effluent, and this injury is exacerbated during episodes of peritonitis [44, 65]. A complete loss of mesothelium following injury, and/or failure of the remesothelialization process, appears to precede end-stage sclerosis [66]. This suggests that in addition to its role in peritoneal host defense, the mesothelium is important in the maintenance of peritoneal membrane structure and function. The observations of increased CA125 concentrations in this study concur with previously reported studies in which solutions specifically manufactured to reduce glucose degradation product (GDP) formation and acidity have a similar impact in vivo (abstract; Simonsen et al, *J Am Soc Nephrol* 10:322A, 1999) [67]. Interestingly, in the former study, switching to the low GDP solution was associated with increased CA125 levels that were subsequently reduced when the patients reverted to acidic lactate-buffered solutions. These data indicate that continuous exposure to conventional solutions is at least partly responsible for the generalized decrease of CA125 (and presumably mesothelial cell mass) over time on PD [23, 67]. While the reduced GDP levels in the solutions (including the B/L solutions used in the present study [12]) are potentially responsible for the observed changes in CA125 (and possibly other markers), further direct evidence is required to substantiate this hypothesis [68, 69]. Reduced GDP levels in solutions have previously been demonstrated to have beneficial effects in vitro, and may offer some biocompatibility advantages in vivo compared with conventional solutions [69, 70].

Continuous dialysis with B/L was associated with decreases in effluent HA levels after both three and six months of treatment. This glycosaminoglycan polymer has many described functions, including participation in angiogenesis, fibrosis, wound healing, tissue remodeling, and inflammation [71, 72]. In the peritoneum, HA is produced by both fibroblasts and peritoneal mesothelial cells [73, 74]. Its synthesis can be stimulated with proinflammatory cytokines such as interleukin (IL)-1β, and its levels correlate with IL-1 and IL-6 levels, indicating its link to ongoing inflammation [73, 74].

Hyaluronic acid has been suggested as a marker of inflammation and tissue remodeling in the peritoneal cavity [21, 28]. In PD patients, HA levels appear to increase with time on PD, suggestive of ongoing and increasing inflammation, wound healing, or mesothelial cell repair processes [21]. The observations made in this study of decreasing HA levels with time of therapy parallel previously reported data and suggest that even following relatively short-term exposure to the B/L solution, decreases in its intraperitoneal levels are observed. This rapid reduction in HA levels following a change to a potentially more biocompatible solution was also observed in our current study (abstract; Simonsen et al, *J Am Soc Nephrol* 10:322A, 1999). As HA contributes to a great number of cellular and inflammatory processes and many of its actions are molecular weight dependent, the absolute meaning of decreased intraperitoneal levels remains to be precisely determined. Previous data demonstrating increasing HA levels with time on PD, however, suggest that these increases parallel changes in the peritoneum (structural/functional) that occur with time on dialysis (abstract; Topley et al, *ibid*) [42, 75, 76]. While

changes in HA may not be directly linked to these processes, reductions in its levels suggest a reduction in intraperitoneal inflammation and a normalization of peritoneal homeostasis. In the present study, the maximal reduction in HA was observed after three months of treatment. It is not clear, however, whether the apparent (but not statistically significant) increase between three and six months represents a reversion of HA levels toward the normal increasing trend seen in conventionally dialyzed patients [21]. The patients enrolled in this study, however, had all been previously dialyzed with conventional solutions for in many cases extended periods. Whether this limits the ability of more biocompatible therapy to reverse the changes previously induced in the peritoneum by conventional dialysis awaits more extended clinical trials comparing the impact of more biocompatible solutions in both new and previously dialyzed patients.

Transforming growth factor-β1 and the PICP were selected as potential markers of fibrosis. TGF-β1 is the prototypical cytokine associated with fibrogenesis in many disease processes throughout the body, capable of inducing extracellular matrix expansion and collagen deposition [29]. PICP and PIIICP have been used as indirect measures of collagen turnover in various tissues, including the peritoneal cavity [26, 30–32]. In the present study, however, there were no changes in either parameter with time in the B/L group or the C group. The observations on collagen turnover concur with the data of Cappelli et al who did not observe significant changes in their cross-over study (when measuring procollagen III C-terminal peptide levels) over an extended treatment period [67]. In contrast, Simonsen et al observed increases in both PICP and PIIICP when patients used the low GDP solution (abstract; Simonsen et al, *ibid*). The reason for the difference between the results presented here and this latter study is unclear, however, the lack of change (and possibly the apparent trend toward a reduction observed in the B/L treatment groups after six months for both TGF-β1 and PICP; data not shown) suggest that there is no indication of increased levels of these potentially profibrotic markers following treatment with the B/L solution. Further studies are clearly warranted to define the natural history of the changes in TGF-β1 and PICP in PD effluent and thus evaluate their link to the process of thickening (including collagen deposition) that occurs in the peritoneal membrane with time on dialysis (abstract; Topley et al, *ibid*).

The two solutions were shown to be therapeutically equivalent with regard to plasma bicarbonate, peritoneal urea, and creatinine clearances [33]. There was a small, but statistically significant increase in 24-hour ultrafiltration in the B/L group (74 mL more over 24 hours, $P < 0.05$). This finding was supported by the six-month peritoneal equilibrium test (PET) ultrafiltration, which was also higher in the treatment group. The increased 24-hour ultrafiltration was not explained by a difference in the concentration of glucose in the bags used by the patients, as the difference remained when the ultrafiltration was normalized for mean glucose concentration [33].

The results of the present study indicate that mesothelial cell mass increases and proinflammatory markers (HA) decrease in the peritoneal effluent of patients exposed to B/L-buffered PD compared with those seen when exposed to conventional acidic, lactate-buffered solutions. These observations complement previous reports of improved in vitro and ex vivo measurements of cell function using identical solution formulations [19, 20, 55]. In evaluating these results, it must also be borne in mind that the patients entering this study had all been previously dialyzed with acidic lactate-buffered solutions, some for extended time periods. In this context, the data indicate (as in other similar studies) that the conversion to a more biocompatible solution is able to reverse the observed decline in CA125 and increase in HA levels that occur with time on PD [21, 23]. While these data do not provide definitive clinical proof of the long-term benefits of conversion to more biocompatible solutions, they do suggest that more biocompatible therapy has a potentially positive (and rapid) impact within the peritoneal cavity. Whether this potential improvement in membrane status will have a long-term impact on deleterious changes in membrane function awaits more extended clinical experience and further planned controlled trials. These observations appear to support the hypothesis that more biocompatible PD solutions will reduce peritoneal inflammation and preserve peritoneal membrane integrity. Both of these actions will hopefully serve to extend successful therapy time on PD.

NOTE ADDED IN PROOF

For further information, the reader is referred to the recently published article: Rippe B, Simonsen O, Heimburger O, *et al:* Long-term clinical effects of a peritoneal dialysis solution with less glucose degradation products. *Kidney Int* 59:348–359, 2001.

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